

“Preliminary Communication on the Œstrous Cycle and the Formation of the Corpus Luteum in the Sheep.” By F. H. A. MARSHALL, B.A. Communicated by Professor J. C. EWART, F.R.S. Received February 15,—Read March 7, 1901.

The sheep employed in this research were for the most part half-breeds between Cheviots or Leicesters and Scotch Black-faced. Some were very kindly kept for me by Professor Ewart at Penycuik, while others were obtained from a neighbouring farmer, and killed at various intervals after copulation. A quantity of material was also obtained from the slaughter-house. In all these breeds the lambs are born in February or March, and the ewes come into season in the following October or November.* Yearling lambs are ready to take the ram about the same time.

Between March and October (period of anœstrum)† the uterus remains in the normal condition (the resting stage). A large number of ovaries from sheep killed in July and August were examined and sections cut, but in no case were there seen either protruding follicles or corpora lutea, or follicles beginning to undergo atresia. Moreover, the walls of the Fallopian tubes showed no sign of congestion of the blood-vessels. Ovaries from sheep killed in the middle of October showed that the follicles were nearly approaching ripeness, this being indicated by the extent of their protrusion, and a little later burst follicles were first observed. From that time to the end of December recently ruptured follicles in sheep's ovaries were quite common. It has been found impossible to draw any hard and fast line between the proœstrum and œstrus for sheep. The latter follows on the proœstrum very quickly, and the two combined are of short duration, probably not more than two days. They will here be considered together, as certain stages which appear to correspond to those which Heape regards as forming part of the proœstrum in other animals occur in sheep at or even after the time of copulation.

At the close of the period of anœstrum certain changes take place in the external reproductive organs, the uterus, and the Fallopian tubes. The vulva becomes distinctly swollen and congested, and I have observed a slight flow of mucus from the external opening, but no blood. Subsequent examination of the uterus has shown that bleeding of the uterine wall is extremely slight, but it is, in some cases at any rate, undoubtedly present. From an examination of the external generative organs it is impossible to determine through what stage of the period of growth or period of degeneration the uterus is passing,

* Dorset sheep alone of British breeds have two gestations a year.

† Heape, “The Sexual Season in Mammals,” ‘Q. J. M. S.,’ vol. 44, November, 1900. The terms “anœstrum,” “diœstrum,” &c., are here explained.

nor has it been, as yet at any rate, possible to state the duration of each or all of these stages. The period of growth is marked by the hypertrophy of the uterine stroma by nuclear division, both in and between the cotyledons. The nuclei in the early stages are distributed most thickly in the region closest to the epithelium of the cotyledons. The blood-vessels increase both in size and number, not at first so much in the cotyledons as between them, and deeper in the stroma and in the muscle layers below the stroma. The uterine cavity, never very large, is at this period almost obliterated. The changes above mentioned result in the breaking down of certain of the blood-vessels. The blood corpuscles thus set free become scattered throughout the stroma, where they form irregularly shaped patches and streaks lying a little below the epithelium, but I have never seen spaces large enough to be described as lacunæ. These corpuscles no doubt go largely to form pigment,* as supposed by Bonnet† and Kazzander.‡ Only in a few places does the epithelium of the cotyledons, as seen in section, lose its continuity, and then not more than four or five cells have disappeared. Passing to such places may be seen small streams of blood corpuscles which were being poured into the uterine cavity. Thus the characteristics of all Heape's stages from I to VI are more or less clearly recognisable.

The sheep, sections through the uterine wall of which show the last-mentioned characters (stage VI), was killed within three hours after coition. A Graafian follicle had just ruptured, as was at once apparent from the bloodstain on its surface, but the blood had not yet clotted. Subsequently cut sections revealed the point of rupture, and also the ovum and discus proligerus, which had not yet been dehiscent. It was apparently from such a case as this that Hausmann§ drew the conclusion that in sheep ovulation cannot take place without coition. That this is not the case, at any rate for the virgin ewe at its first œstrus, I subsequently proved. Some yearling lambs were kept along with a ram which was rendered temporarily incapable of insemination by the method generally followed by sheep breeders. The time when the ewes came into season was indicated by their attitude towards the ram. Œstrus having been detected by this means, the ewe in

* Black pigment may not infrequently be observed, especially between and round the bases of the cotyledons, beneath the uterine epithelium. In one case the pigment was so distributed as to render the interior of the uterus perfectly black between the cotyledons. I have never observed this pigment in the uterus of yearling lambs.

† Bonnet. See Ellenberger's '*Vergleichende Physiol. d. Haussäugethiere*,' vol. 2, Berlin, 1892.

‡ Kazzander, "*Über d. Pigmentation d. Uterinschleimhaut des Schafes*," '*Arch. f. Mikr. Anat.*,' vol. 36, 1892.

§ Hausmann, '*Ueber die Zeugung und Entstehung des wahren weiblichen Eies*,' &c., Hanover, 1840.

question was separated from the rest, and a day afterwards killed, when it became evident at once from the blood-clot on the surface of one of the ovaries that ovulation had recently taken place. Sections through this ovary showed the point of rupture of the follicle. This fact, that ewes need not be served in order to induce ovulation, is of considerable importance, as it indicates the possibility of obtaining successful results from the artificial insemination of sheep.

When ovulation takes place, one follicle only may rupture at a time, or one follicle in each ovary, or two in the same ovary. I have never observed any greater number of discharged follicles of the same age in the ovaries of a sheep.*

The period of "heat" in sheep is further marked by the distension of the blood-vessels of the Fallopian tubes, which may throughout almost their entire length be coloured a deep purple. The increased size of the vessels is also seen in section, but there is no breaking down of vessels. There is too some evidence of increased blood supply to the ovaries, apart from the region of the ruptured follicle.

The changes which take place in the metœstrous period have not as yet been fully worked out, but at a period three days after coition, red blood corpuscles in a state of hæmorrhage, and arranged in streaks below the epithelium, have been observed. It would also appear that new capillaries have been formed. Metœstrum is succeeded by a period of rest (diœstrum), which after not many days is followed by another proœstrum, and so on, until the sheep becomes pregnant or the breeding season is over. The complete diœstrous cycle in the sheep in the only case which came under my observation was fifteen days, but from the observations of others with whom I have spoken it would appear to vary from about thirteen to eighteen days.

The Formation of the Corpus luteum.—The age of the corpus luteum in this investigation was in each case reckoned, either from copulation, or, where copulation did not or was not known to have taken place, from the time when œstrus was observed. Of course it is possible that ovulation does not always take place during œstrus, but the observed relation between the state of development of the corpus luteum and the time that had elapsed between œstrus and the killing of the animal is by itself strong evidence that in the sheep the two phenomena are approximately coincident. In no case after a sheep in which œstrus had been observed, was killed to obtain a stage in the development of the corpus luteum, was the corpus luteum not found. It could usually be at once readily detected by the blood-clot which remains on the surface of the ovary for several days after the rupture of the follicle.

The corpus luteum of seven hours differs from the unburst follicle in its size and in the fact that the ovum and discus proligerus have

* Triplets are, however, not uncommon in some breeds of sheep.

been discharged. It is rather more than half as large as the ripe follicle, and consequently does not protrude from the surface of the ovary. Very little blood remains within the cavity, but corpuscles are seen scattered through the membrana granulosa, these being derived from vessels whose walls have broken down, not only near the point of rupture of the follicle, but to a less extent around the whole theca interna. The membrana granulosa is approximately twice the thickness of that of the ripe follicle, some of the cells having increased largely in size, while others, especially those nearest to the periphery, retain the characters of the original follicular epithelial cells. The central cavity contains a fluid resembling in all respects the liquor folliculi. At this stage there is no sign of any growth inward of the theca interna, and I have not observed any mitoses among the cells of that layer.

The corpus luteum of twenty-four hours has undergone considerable changes. Its increase in size is well marked, its dimensions now approaching those of the ripe Graafian follicle. Its shape is generally irregular, and its walls are much folded. The central cavity is smaller. This cavity, which, as in the earlier stage, contains a fluid, communicates with the exterior by a slit-like passage opening into a cup-shaped depression on the surface of the ovary, from which the corpus luteum now appreciably protrudes. The depression and slit-like passage represent the point of rupture of the follicle. The epithelial wall of the cavity is at this period at least twice as thick as that of the seven-hour stage, this increase being due for the most part to the simple hypertrophy of the individual cells composing it, these appearing in section two or three times the size of those of the membrana granulosa of the Graafian follicle. Division is, however, not very infrequently to be observed among the epithelial cells. But the thickness of this layer is also increased by the ingrowth of connective tissue, strands of which, arising by cell proliferation of the theca interna, are growing inwards and penetrating the epithelium. These connective tissue strands present a radial appearance. The cells of which they are composed are commonly fusiform in shape, and mitotic division is very common among them. But although the connective tissue element of the corpus luteum of the sheep is provided chiefly by the proliferation of the cells of the theca interna, it is in part derived from the more fibrous theca externa, from which layer strands of cells, usually in close connection with those of the inner layer, are at this stage beginning to grow inwards between the epithelial cells. Red blood corpuscles occur in scattered patches and streaks, as in the earlier stage.

In the corpus luteum thirty hours after coition, the inner theca layer has all but disappeared, having been used up in the formation of the inter-epithelial connective tissue. The epithelial cells, which have still

further hypertrophied, are now in places surrounded by a network of fusiform cells. The point of rupture of the follicle is still open, and communicates with the fluid-containing cavity.

The epithelial cells of the corpus luteum of about fifty hours are four or five times the size of those of the undischarged follicle, as seen in section. Mitotic division is very rare among them, but evidence of it may still occasionally be observed. Proliferation of the connective tissue cells continues to take place, chiefly in the direction of the central cavity, which has become smaller. Leucocytes are to be seen among the epithelial cells, as well as free red corpuscles. The inner theca layer, as such, has disappeared. The corpus luteum as a whole presents a radial appearance.

The corpus luteum of sixty hours has undergone a further change. The connective tissue cells are dividing in all directions, so that nearly every epithelial cell is surrounded by an anastomosis of fusiform cells. The central cavity also is completely enclosed by a layer of connective tissue. The epithelial cells are still increasing in size by simple hypertrophy, but I have not observed any case of division. Large blood-vessels, derived from those of the inner theca, may be seen in the epithelium near the periphery. The corpus luteum is now larger than the ripe follicle.

The succeeding stages in the development of the corpus luteum show the still further increase in the connective tissue proliferation, and in the hypertrophy of the epithelial cells, and the consequent growth in size of the whole structure. The dimensions of the developing corpus luteum are, however, no sure guide to its age, for I have observed two in the same ovary and of the same age, but with an appreciable difference in size. Blood vessels, at first only to be observed near the theca interna, spread towards the centre. The cavity becomes obliterated by the inward growth of connective tissue, and the point of rupture ceases to be visible. The connective tissue becomes more and more finely distributed throughout the epithelium. When the cells of the latter have attained a size of about six times the dimensions of those of the unaltered membrana granulosa of the ripe follicle, fatty degeneration sets in, and they become converted into lutein cells.

The above account of the development of the corpus luteum in the sheep agrees substantially with that given by Sobotta* for the mouse and the rabbit, and by Stratz† for *Tupaia* and *Tarsius*. It differs from Sobotta in the description of the part played by the theca externa, and in recording the not infrequently observed multiplication of the ep-

* Sobotta, "Ueber die Bildung des Corpus luteum bei der Maus," 'Archiv f. Mikr. Anat.' vol. 47, 1896; "Ueber die Bildung des Corpus luteum beim Kaninchen, &c.," 'Anatomische Hefte,' vol. 8, 1897.

† Stratz, 'Der geschlechtsreife Säugethiereierstock,' Haag, 1898.

thelial cells by mitotic division in the earlier stages. In the latter respect it tends to agree with Belloy,* who, however, describes active multiplication of these cells, and does not appear to recognise the proliferation of the cells of the theca interna. On the other hand the foregoing account is entirely opposed to the views expressed since the publication of Sobotta's work by His,† Koelliker,‡ Nagel,§ Clark,§ Rabl,|| and Doering,¶ who all more or less clearly describe the corpus luteum as an entirely connective-tissue structure.

It remains to be added that the work is being carried on in the Zoological Department of the University of Edinburgh.

[*Postscript, February 22.*—Since writing the above account of the development of the corpus luteum, I have obtained a 16-hour stage from a sheep which was an unusually late breeder. The characters of the young corpus luteum at this stage are intermediate between those of the 7-hour and 24-hour stages. The inward growth of the connective-tissue cells has begun. The sections pass through the point of rupture, which is widely open.]

“On the Composition and Variations of the Pelvic Plexus in *Acanthias vulgaris*.” By R. C. PUNNETT, B.A., Gonville and Caius College, Cambridge. Communicated by Dr. H. GADOW, F.R.S. Received February 16,—Read March 7, 1901.

(Abstract.)

The facts recorded in this paper may be summarised as follows :—

(1) Considerable variation occurs in *Acanthias vulgaris* with regard to—

- (a) The serial number of the girdle-piercing nerves ;
- (b) The number of the post-girdle nerves ;

* Belloy, “Recherches sur l'origine des corps jaune de l'ovaire chez le rat et le cochon d'Inde,” ‘Comptes Rendus de l'Association des Anatomistes,’ publiés par le Professeur A. Nicolas, première session, Paris, 1899.

† His, } “Verhandlungen der Anatomischen Gesellschaft auf der zwölften
Koelliker, } Versammlung in Kiel,” April, 1898, ‘Anat. Anz.,’ vol. 14.

‡ Nagel, “Die weiblichen Geschlechtsorgane,” ‘Handbuch der Anatomie des Menschen,’ herausg. von K. v. Bardeleben, bd. 7, teil 2, abt. 1, Jena, 1896.

§ Clark, “Ursprung, Wachstum und Ende des Corpus luteum, &c.,” ‘Arch. f. Anat. u. Physiol., Anat. Abth.,’ 1898.

|| Rabl, “Beitrag zur Histologie des Eierstocks,” &c., ‘Anat. Hefte,’ vol. 11, 1898.

¶ Doering, “Beitrag zur Streitfrage über die Bildung des Corpus luteum,” ‘Anat. Anz.,’ vol. 16, 1899.